PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 94/10125
C07C 69/587, A61K 31/23	A1	(43) International Publication Date:	11 May 1994 (11.05.94)

(21) International Application Number: PCT/EP93/02917

(22) International Filing Date: 21 October 1993 (21.10.93)

(30) Priority data:

4/288684	27 October 1992 (27.10.92)	JP
4/310253	19 November 1992 (19.11.92)	JP
5/176750	16 July 1993 (16.07.93)	JP
5/204182	18 August 1993 (18.08.93)	JP

(71) Applicant *(for AT only)*: SANDOZ-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).

(71) Applicant *(for DE only)*: SANDOZ-PATENT-GMBH [DE/DE]; Humboldstrasse 3, D-79539 Lörrach (DE).

(71) Applicant *(for all designated States except AT DE US)*: SANDOZ LTD. [CH/CH]; Lichtstrasse 35, CH-4002 Basle (CH).

(72) Inventors; and

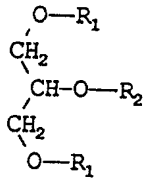
(75) Inventors/Applicants *(for US only)* : NAGAHAMA, Shizuo [JP/JP]; 2-28-210, Kusaba-cho, Kumamoto-shi (JP). OCHIAI, Keiko [JP/JP]; 1847-234, Oomuro, Kashiwa-shi, Chiba-ken (JP). OHBA, Setsuya [JP/JP]; 2-4-1-404, Amakubo, Tsukuba-shi, Ibaraki-ken (JP). TOMITA, Takako [JP/JP]; 1036-36, Kusanagi, Shimizu-shi, Shizuoka-ken (JP). WAKABAYASHI, Toshio [JP/JP]; 4-6-3-512, Ninomiya, Tsukuba-shi, Ibaraki-ken (JP).

(74) Common Representative: SANDOZ LTD.; Patents & Trademarks Div., Lichtstrasse 35, CH-4002 Basle (CH).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: GLYCERIN DERIVATIVES AND USES THEREOF



(I)

(57) Abstract

A compound of formula (I) wherein R_1 and R_2 are acyl groups derived from different unsaturated fatty acids, both R_1 groups being identical, one acyl group of R_1 and R_2 is an acyl group derived from eicosapentaenoic acid or docosahexaenoic acid, and theother acyl group is an acyl group derived from linoleic acid, γ-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid, and which is preferably in pure form comprising at least 90 % of the compound, has platelet aggregation inhibition activity and can be effectively used not only as a medicine for the therapy or prevention of thrombotic inflammation and platelet aggregation-induced arterial sclerosis, but also as a well-balanced nutrient clysis. Also certain compounds of formula (I), in particular the compound in which R_1 is an eicosapentaenoyl residue and R_2 is a γ-linolenoyl residue, have anti-hypertriglyceridemic activity and are useful for the therapeutic treatment or prevention of diseases caused by hypertriglyceridemic such as cardiac infarction or arteriosclerosis.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

Glycerin Derivatives and Uses Thereof

The present invention relates to glycerin derivatives, in particular to triglycerides, and pharmaceutical and nutrition uses thereof.

It is known that certain unsaturated fatty acids including eicosapentaenoic (EPA), docosahexaenoic acid (DHA) and γ-linolenic acid (GLA) play an important role in prostaglandin synthesis and have antithrombotic and hypolipaemic activities. Also glycerides of unsaturated fatty acids have been proposed for use in medicine or nutrition: for prevention and treatment of gallstones (Japanese patent application J6 0169-148A - Nippon Oils & Fats KK); for antithrombotic and hypolipaemic uses (European Patent Application EP 0298293 - Fresnius AG); to improve peripheral blood circulation (European Patent Application EP 0304603), and for prevention of thromboses (Japanese patent application J6 0132-916A - Nisshin Oil Mills KK). Also a fat clysis preparation containing purified soybean oil is commercially available as Soyacal (GB 2050799 - Green Cross Corporation).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are unsaturated fatty acids contained in fish oils of saltwater fish such as sardine, mackerel and saury. These fish oils contain complex mixtures of numerous fatty acid triglycerides.

Linoleic acid (LLA) is an unsaturated fatty acid component of plant oils such as soybean oil and sunflower oil, and γ-linolenic acid (GLA) is an unsaturated fatty acid contained in plant oils such as evening primrose oil. These plant oils are also composed of complex mixtures of numerous fatty acid triglycerides.

The above plant oils contains neither EPA nor DHA. Consequently

single compound triglyceride products containing EPA and/or DHA in combination with LLA and/or GLA are not natural components of plant oils, or fish oils nor can they be separated or purified therefrom.

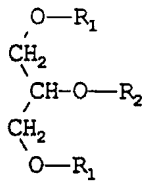
Purified soybean oil is composed of triglycerides containing fatty acids which have up to 18 carbon atoms. On the other hand, human cells contain lipid derived from unsaturated fatty acids having at least 20 carbon atoms such as EPA and DHA, particularly DHA. Thus fat clysis preparations based on purified soybean oil cannot effectively provide the eicosapentaenoic acid and docosahexaenoic acid required by human cells.

The unsaturated fatty acid triglycerides which have been used to date in medicines and foods have comprised complex mixtures of many different triglyceride compounds. It is believed that single, chemically defined triglyceride compounds will provide improved medicinal and food products.

We have synthesized symmetrical triglycerides and have provided purified single compound products containing eicosapentaenoic acid or docosahexaenoic acid and having acyl groups derived from identical unsaturated fatty acids on the 1-position and 3-position of glycerol and have found that these triglyceride products have platelet aggregation inhibition activity and are also useful as a nutrient clysis. Also we have found that certain of the compounds that we have synthesised have anti-hypertriglyceridemic activity.

Thus the objects of the present invention include the provision of novel triglyceride derivatives, and platelet aggregation inhibitor, fat clysis and anti-hypertriglyceridemic preparations containing the derivatives:

Accordingly the present invention provides a compound of formula (I).



I

wherein R_1 and R_2 are acyl groups derived from different unsaturated fatty acids, both R^1 groups being identical, one acyl group of R_1 and R_2 is an acyl group derived from EPA or DHA, and the other acyl group is an acyl group derived from LLA, GLA, EPA or DHA.

In particular the invention includes a compound of formula I in pure form. For the purposes of the present description a compound of formula I "in pure form" comprises at least 90%, preferably at least 95%, especially about 96-99%, by weight of a single compound of formula I. Such a compound in pure form typically contains less than 10%, advantageously less than 5%, by weight in total of other compounds of formula I.

Specific examples of compounds of formula (I) will be apparent from the definitions of R_1 and R_2 in the above formula (I) and compounds in Examples to be described later.

The compounds of formula I may be chemically synthesized; for instance in a first embodiment, as described below.

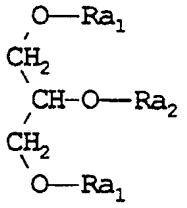
In a first step, glycerol is allowed to react with LLA, GLA, EPA or DHA to obtain the corresponding 1,3-diacylglyceride. The amount of unsaturated fatty acid used per mole of glycerol is preferably 1.9 to 2.2 mol. The reaction temperature is preferably between -30°C and -10°C. The solvent is selected preferably from pyridine, tetrahydrofuran, methylene chloride and mixtures of these. The condensation agent used for the condensation reaction is preferably N,N'-dicyclohexylcarbodiimide.

In a second step, the above 1,3-diacylglyceride is reacted with an unsaturated fatty acid to provide a 1,2,3,-triacylglyceride of formula (I). The unsaturated fatty acid used in the second step is different from that used in the first step and is EPA or DHA, when LLA or GLA is used in the first step; EPA LLA or GLA, when DHA is used in the first step; or DHA LLA or GLA when EPA is used in the first step. Preferably N,N'-dicyclohexylcarbodiimide is used as the condensation agent in the second step. The amount of unsaturated fatty acid used per mole of the 1,3-diacylglyceride is preferably 0.95 to 1.1 mol. The reaction temperature is preferably room temperature, and the reaction solvent is selected from methylene chloride, ethyl acetate and tetrahydrofuran.

Alternatively in a second embodiment the 2-monoacylglyceride may be prepared in a first step and this intermediate then reacted to provide the triglyceride of formula I.

Dimethylaminopyridine is preferably used as a reaction catalyst in both the first and second steps.

Thus the invention includes a process for the production of a compound of formula I which comprises appropriately acylating a compound of formula Ia



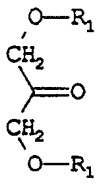
Ia

wherein Ra_1 is R_1 or hydrogen, both Ra_1 groups are identical and Ra_2 is R_2 or hydrogen, provided that one of Ra_1 and Ra_2 is other than hydrogen.

Alternatively in a preferred embodiment the compound of formula I may be prepared using dihydroxyacetone ($HOCH_2COCH_2OH$) as

starting material, comprising 1) a first step in which the dihydroxyacetone is reacted with LLA, GLA, EPA or DHA to obtain the corresponding 1,3-diacyl-2-propanone; 2) a second step in which the 2-propanone is reduced to give the corresponding 1,3-diacylglyceride; and 3) a third step in which the 1,3-diacylglyceride is reacted with an unsaturated fatty acid to provide a 1,2,3-triacylglyceride of formula I, as described above for the second step of the first embodiment.

Accordingly the invention includes a process for the production of a compound of formula I, which comprises the step of reducing a 1,3-diacyl-2-propanone of formula Ib



Ib

where in R_1 is as defined above, to obtain the corresponding 1,3-diacylglyceride.

The compounds of formula I have pharmacological activity and are therefore useful as pharmaceuticals.

In particular the compounds show platelet aggregation inhibition activity; for instance when tested in an assay as described in Example 11. The compounds are, therefore, useful for the therapy or prevention of diseases caused by platelet aggregation such as thrombotic inflammation and arterial sclerosis.

Also the compounds may also be used in a well-balanced nutrient clysis.

Moreover particular compounds of formula I, especially the compound of formula IX as given hereinafter in Example 8 has anti-hypertriglyceridemic activity; for instance when tested in

an assay as described in Example 12. Compounds having such anti-hypertriglyceridemic activity are useful for the therapeutic treatment or prevention of diseases caused by hypertriglyceridemia, such as cardiac infarction or ateriosclerosis.

Thus the invention also includes the use of a compound of formula I, e.g. in pure form, as a pharmaceutical or nutrient.

Further the invention includes a pharmaceutical or nutrient, e.g. fat clysis, composition comprising an effective amount of a compound of formula I, e.g. in pure form.

Such compositions typically comprise the compound of formula I together with a pharmaceutically- or nutritionally-acceptable diluent, excipient or vehicle. The compositions may be for oral or parenteral, including injectable, administration. The dose at which the compound is administered to adults, both for inhibition of patelet aggregation and anti-trihypertriglyceridemic uses, may be in the range from 100 mg to 5 g/day, preferably from 200 mg to 2 g/day. It can be administered once a day or by dividing a dose into two or three doses a day as required. It is preferably orally administered or intravenously injected.

When a preparation for oral administration is produced, the compound of the present invention may be prepared in capsule, tablet or granular form by mixing it with a preparation vehicle or excipient according to a usual method. Further, the compound of the present invention can be included with cyclodextrin to stabilize it. For intravenous injection, the triglyceride derivative of the present invention can be prepared as an emulsion by dispersing the triglyceride derivative, normally at a concentration in the range from about 10 to about 20% (w/v), with distilled water for injection, purified yolk lecithin and glycerin and emulsifying the resultant dispersion under pressure. When an emulsion is prepared purified soybean oil may be mixed with the triglyceride derivative. In this case, the mixing ratio

of the purified soybean oil is preferably in the range of 0 to 97 w/v%.

The fat clysis preparation of the present invention can be prepared by the same method as that used for preparing the above emulsion for intravenous injection.

The fat clysis preparation according to the invention can be used not only as a clysis preparation for the therapy of thrombotic inflammation and arterial sclerosis, but also as a well-balanced nutrient clysis.

The dose at which the emulsion may be injected into adults, both when used for therapy and as a nutrient clysis, is usually in the range from 1 ml to about 500 ml per day.

Among the compounds of the formula (I), compounds II, V, VII and X, particularly compounds II, V and X, as described in Examples 1, 4, 6 and 9, are preferred for use in fat clysis preparations.

The invention is further illustrated in, though not limited by, the following Examples nos. 1 to 13.

EXAMPLES

Example 1 Compound of formula I wherein R_1 is linoleyl residue and R_2 is docosahexanoyl residue

(1) 8 ml of tetrahydrofuran, 30 mg (0.24 mM) of 4-dimethylaminopyridine and 1,083 mg (5.21 mM) of, N,N'-dicyclohexylcarodiimide were added to 242 mg (2.63 mM) of glycerin, to which a solution of 1,473 mg (5.26 mM) of linoleic acid in 4 ml of methylene chloride is then added dropwise under a nitrogen atmosphere at -20°C. The resultant mixture is allowed to react for 22 hours while the reaction temperature is maintained between -30°C and -10°C. The reaction mixture is then filtered, and the filtrate evaporated to dryness under reduced pressure. The residue is subjected to silica gel chromatography, and 441 mg of 1,3-dilinoleylglyceride is obtained from a methylene chloride-acetone (98.5:1.5) elution fraction. This product has the following physiocochemical characteristics.

IRν KBr Max (cm^{-1}): 3480, 1744

NMR (CDCl3), δ(ppm): 0.89 (6H, t, J = 7.0 Hz), 5.35 (8H,m)

MASS (m/e): 616 (M^+) puritiy >98%

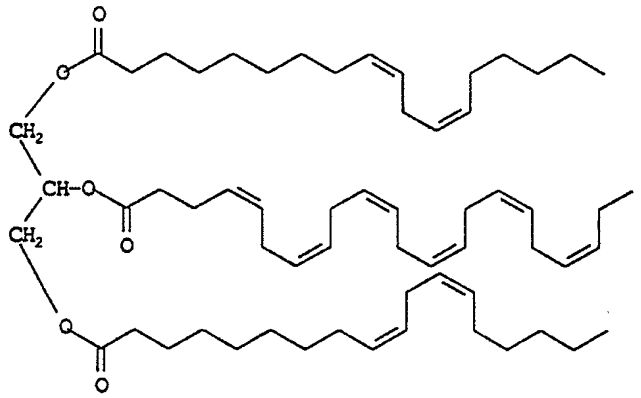
(2) 3 ml of methylene chloride is added to a mixture of 307 mg (0.49 mM) of the 1,3-dilinoleylglyceride, 10 mg (0.08 mM) of 4-dimethylaminopyridine and 101 mg (0.49 mM) of N,N'-dicyclohexylcarbodiimide, to which a solution of 168 mg (0.51 mM) of docosahexaenoic acid in 1 ml of methylene chloride is then added dropwise at room temperature under a nitrogen atmosphere. The resultant mixture is allowed to react at room temperature overnight, the reaction mixture is then filtered, and the filtrate is evaporated to dryness under reduced pressure. Then, the residue is subjected to silica gel chromatography, and 422 mg of 1,3-di-9,12-octadecadienoyl-2-4,7,10,13,16,19

docosahexaenoylglyceride is obtained from a methylene chloride elution fraction. This product has the following physicochemical characteristics.

IRν Max (neat) (cm^{-1}): 3015, 1746 puritiy >95%

NMR ($CDCl_3$), δ(ppm): 0.89 (6H, t, J = 7.0 Hz), 0.97 (3H, t, 7.2 Hz), 5.38 (21H, m)
MASS (m/e): 926 (M^+), 647, 599

The above physicochemical data are consistent with a structure of formula (II).



II

Example 2 Compound of formula I wherein R_1 is linoleyl residue and R_2 is eicosapentaenoyl residue

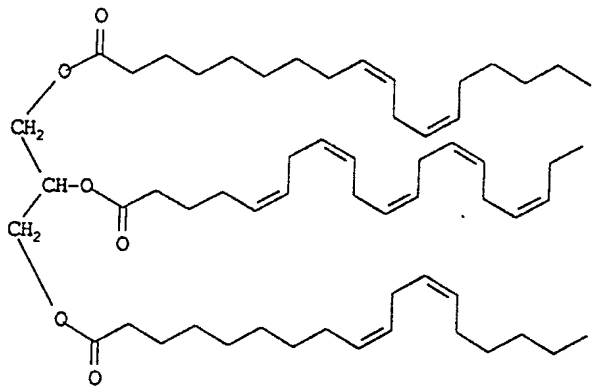
Eicosapentaenoic acid is allowed to condensation-react with 1,3-dilinoleylglyceride as obtained in Example 1(1) in the same manner as in Example 1(2) to give 1,3-di-9,12-octadienoyl-2-5,8,11,14,17-eicosapentaenoylglyceride. This product has the following physicochemical charcteristics.

IRν Max (KBr) (cm^{-1}): 3016, 1746 puritiy >95%

NMR (CDCl3), δ(ppm): 0.89(6H, t, J = 7.0 Hz), 0.97 (3H, t, 7.6 Hz), 5.35 (19H, m)

MASS (m/e): 900 (M^+), 599

The above physicochemical characteristics are consistent with a structure of formula (III).



III

Example 3 Compound of formula I wherein R_1 is γ-linoleyl residue and R_2 is docosahexaenoyl residue

(1) 0.8 ml of pyridine, 6 ml of tetrahydrofuran, 35 mg (0.29 mM) of 4-dimethylaminopyridine and 741 mg (3.56 mM) of N,N'-dicyclohexylcarbodiimide is added to 172 mg (1.87 mM) of glycerin, to which a solution of 1 g (3.60 mM) of γ-linoleic acid in 3 ml of methylene chloride is then added dropwise under a nitrogen atmosphere at -20°C. The resultant mixture is allowed to react for 22 hours while the reaction temperature is maintained between -30°C and -10°C. The reaction mixture is filtered, and the filtrate evaporated to dryness under reduced pressure. The residue is then subjected to silica gel chromatography, and 701 mg of 1,3-diγ-linolenoylglyceride is obtained from a methylene chloride-acetone 1% elution fraction. This product has the following physicochemical characteristics.

IRν KBr Max (cm^{-1}): 3480, 1744 purity >98%

NMR (CDCl3), δ(ppm): 0.89 (6H, t, J = 7.0 Hz), 5.36 (12H,m)

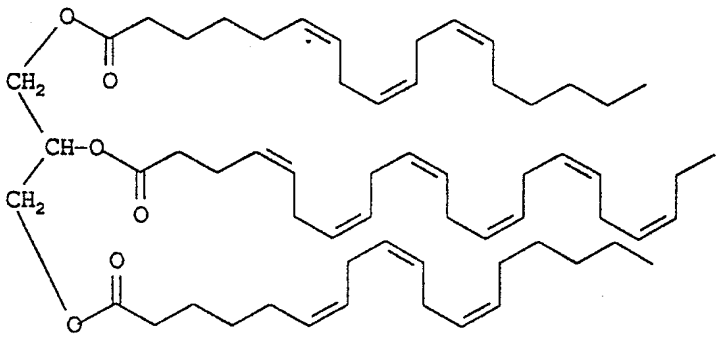
MASS (m/e): 612 (M^+)

(2) 1.5 ml of methylene chloride is added to a mixture of 219 mg (0.36 mM) of the 1,3-diγ-linolenoylglyceride, 10 mg (0.08 mM) of 4-dimethylaminopyridine and 76 mg (0.37 mM) of N,N'-dicyclohexylcarbodiimide, to which a solution of 121 mg (0.37 mM) of docosahexaenoic acid in 1 ml of methylene chlordie is then added dropwise at room temperature under a nitrogen atmosphere. The resultant mixture is allowed to react at room temperature overnight, and then the reaction mixture is filtered. The filtrate evaporated to dryness under reduced pressure. The residue is subjected to silica gel chromatography, and 295 mg of 1,3-di-6,9,12-octadecatrienoyl-2-4,7,10,13,16,19-docosahexenoylglyceride is obtained from a methylene chlorîde-hexane (1:1) elution fraction.

IRν_{Max}^{KBr} (cm^{-1}); 3016, 1746 purity >96%

NMR ($CDCl_3$), δ(ppm): 0.89 (6H, t, J = 7.0 Hz), 0.97 (3H, t, 7.4 Hz), 5.37 (25H, m)
MASS (m/e): 922 (M^+), 645, 595

The above physicochemical data are consistent with a structure of formula (IV).



IV

Example 4 Compound of formula I wherein R_1 is docosahexaenoyl residue and R_2 is linoleyl residue

(1) Glycerin and docosahexaenoic acid are allowed to condensation-react in the same manner as in Example 1(1) to obtain 1,3-didocosahexaenoylglyceride. This product has the following physicochemical characteristics.

IRν KBr Max (cm^{-1}): 3466, 3018, 1742 purity >97%

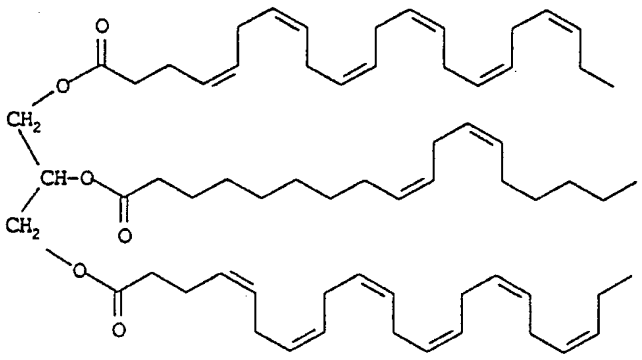
NMR ($CDCl_3$), δ(ppm): 0.97 (6H, t, J = 7.0 Hz), 5.37 (24H, m)
MASS (m/e): 712 (M^+), 385

(2) The above 1,3-didocosahexaenoylglyceride is allowed to condensation-react with linoleic acid in the same manner as in Example 1(2) to give 1,3-di-4,7,10,13,16,19-docosahexaenoyl-2-9,12-octadecadienoylglyceride. This product has the following physicochemical characteristics.

IRν KBr Max (cm^{-1}): 3018, 1746 purity >96%

NMR ($CDCl_3$), δ(ppm): 0.97 (9H, t, J = 7.5 Hz), 5.37 (29H, m)
MASS (m/e): 974 (M^+), 647

The above physicochemical data support a structure of formula (V).



V

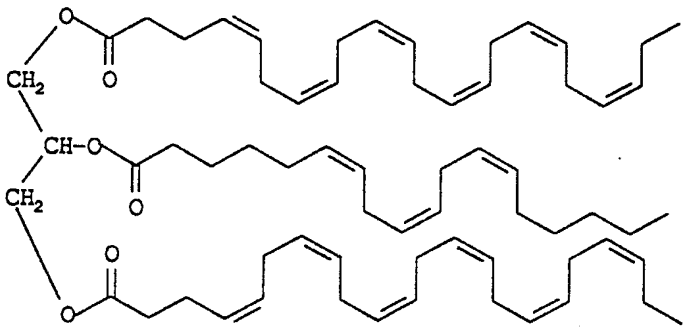
Example 5 Compound of formula I wherein R_1 is docosahexaenoyl residue and R_2 is γ-linolenyl residue

1,3-Didocosahexaenoylglyceride as obtained in Example 4(1) is allowed to condensation-react with γ-linolenic acid in the same manner as in Example 1(2) to give 1,3-di-4,7,10,13,16,19-docosahexaenoyl-2-6,9,12-octadecatrienoylglyceride. This product has the following physicochemical characteristics.

IRν KBr Max (cm^{-1}): 3016, 1740 purity >95%

NMR ($CDCl_3$), δ(ppm): 0.86 (9H, t, J = 7.0 Hz), 5.32 (31H, m)
MASS (m/e): 972 (M^+), 695

The above physicochemical are consistent with a the structure of formula (VI).



VI

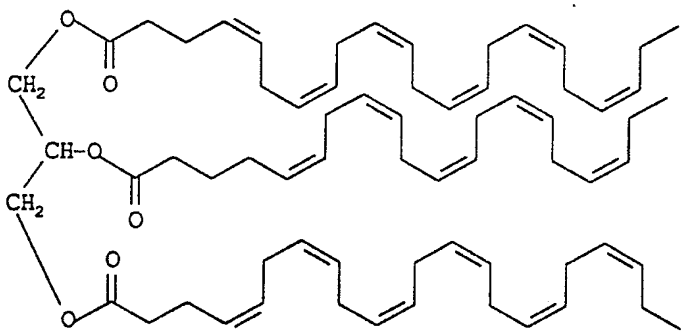
Example 6 Compound of formula I wherein R_1 is docosahexaenoyl residue and R_2 is eicosapentaenoyl residue

1,3-Didocosahexaenoylglyceride is allowed to condensation-react with eicosapentaenoic acid in the same manner as in Example 4(2) to give 1,3-di-4,7,10,13,16,19-docosahexaenoyl-2-5,8,11,14,17-eicosapentaenoylglyceride.
This product has the following physicochemical characteristics:

IRν (neat, Max) (cm^{-1}): 3015, 1745 purity >94%

NMR ($CDCl_3$), δ(ppm): 1.00 (9H, t, J = 7.3 Hz), 5.40 (35H, m)
MASS (m/e): 996 (M^+), 695, 669

The above physicochemical data are consistent with a structure of formula (VII).



VII

Example 7 Compound of formula I wherein R_1 is eicosapentaenoyl residue and R_2 is linoleyl residue

(1) Glycerin and eicosapentaenoic acid are allowed to condensation-react in the presence of the same solvent as that used in Example 3(1) to obtain 1,3-dieicosapentaenoylglyceride. This product has the following physicochemical characteristics.

IRν (KBr, Max) (cm^{-1}); 3470, 3016, 1742 purity >97%

NMR ($CDCl_3$), δ(ppm): 0.97 (6H, t, J = 7.4 Hz), 5.34 (20H, m)
MASS (m/e): 660 (M^+), 359

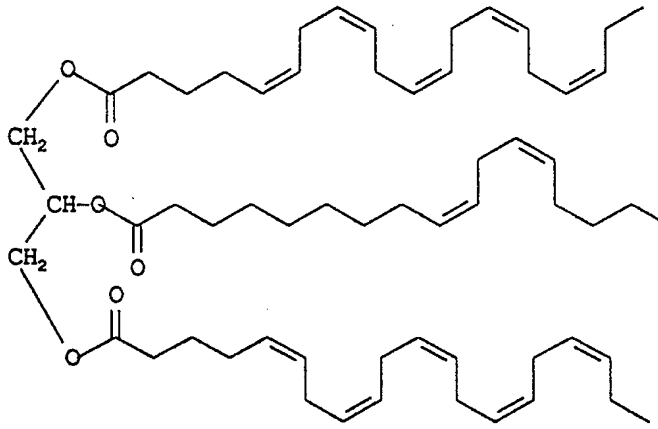
(2) The above 1,3-dieicosapentaenoylglyceride is allowed to react with linoleic acid in the same manner as in Example 1(2) to give 1,3-di-5,8,11,14,17-eicosapentaenoyl-2-9,12-octadecadienoyl

glyceride. This product has the following physicochemical characteristics.

IRν Max (neat) (cm^{-1}): 3015, 1745 purity ~96%

NMR ($CDCl_3$), δ(ppm): 0.86 (3H, t, J = 7.0 Hz), 0.97 (6H, t, 7,3 Hz), 5.25 (25H, m)
MASS (m/e): 922 (M+), 621

The above physicochemical data support a structure of formula (VIII)



VIII

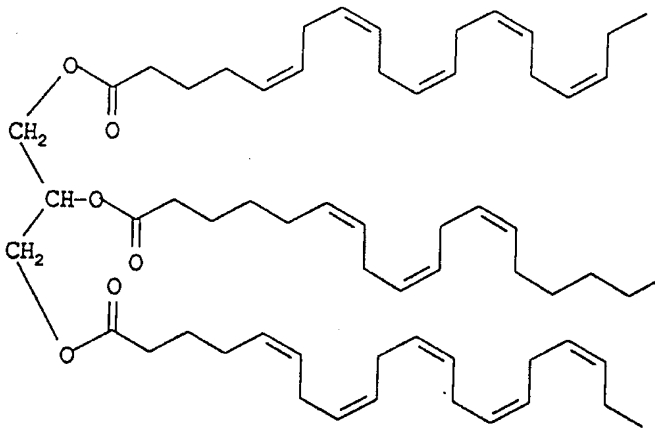
Example 8 Compound of formula I wherein R_1 is eicosapentaenoyl residue and R_2 is γ-linolenyl residue

1,3-Dieicosapentaenoylglyceride as obtained in Example 7(1) is allowed to react with γ-linolenic acid in the same manner as in Example 1(1) to obtain 1,3-dieicosapentaenoyl-2-6,9,12-octadecatrienoylglyceride. This product has the following physicochemical characteristics.

IRν Max (KBr) (cm^{-1}); 3016, 1746 purity >96%

NMR ($CDCl_3$), δ(ppm): 0.89 (3H, t, J = 7.0 Hz), 0.97 (6H, t, 7,4 Hz), 5.37 (27H, m)
MASS (m/e): 920 (M^+), 619

The above physicochemical data support a structure of formula (IX).



IX

Example 9 Compound of formula I wherein R_1 is eicosapentaenoyl residue and R_2 is docosahexaenoyl residue

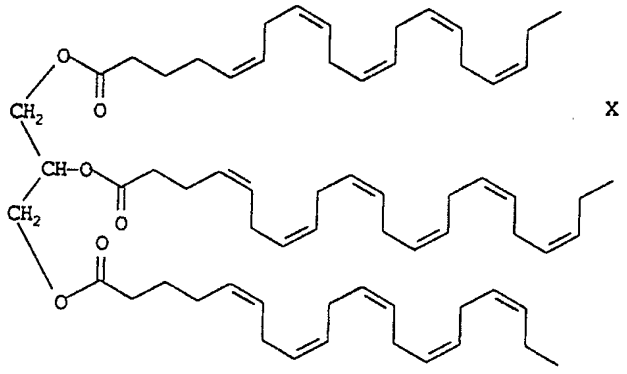
1,3-Dieicosapentaenoylglyceride as obtained in Example 7(1) is allowed to react with docosahexaenoic acid in the same manner as in Example 1(2) to obtain 1,3-dieicosapentaenoyl-2-4,7,10,13,16,19-docosahexaenoylglyceride. This product has the following physicochemical characteristics.

IRν_{Max}^{KBr} (cm^{-1}); 3016, 1746 purity >94%

NMR ($CDCl_3$), δ(ppm): 0.96 (9H, t, J = 7.3 Hz), 5.37 (33H, m)

MASS (m/e): 970 (M^+), 669, 643

The above physicochemical data support a structure of formula (X).



X

Example 10 Alternative Process for the preparation of the compound of formula I wherein R_1 is eicosapentaenyl residue and R_2 is linolyl

(1) A solution of 3.02 g (10mM) of eicosapentaenoic acid in 10 ml of methylene chloride is prepared, and this solution is added to a solution of 1.78 g (11mM) of carbonyl diimidazole in 10 ml of methylene chloride under an atmosphere of nitrogen. The mixture is allowed to react at room temperature for 3 hours. Then 450 mg (2.5 mM) of a dihydroxyacetone dimer and 1.2 g (10mM) of 4-dimethylaminopyridine are added, and the resultant mixture is allowed to react at room temperature overnight. The reaction mixture is washed with water and evaporated to dryness under reduced pressure. The residue is subjected to silica gel column chromatography, and 2.70 g of 1,3-bis-5,8,11,14,17-eicosapentaenoyl-2-propanone is obtained from the methylene chloride elution fraction. The physicochemical characteristics of this product are as follows.

$IR\upsilon_{max}^{KBr}$ (cm^{-1}): 3016, 1745

NMR ($CDCl_3$), δ(ppm) : 0.97(6H,t,J=7.0Hz), 4.76(4H,s), 5.41(2OH,m)

(2) 2.64 g (4.0 mM) of the 1,3-bis-5,8,11,14,17-eicosapentaenoyl-2-propanone obtained in (1) is dissolved in 20 ml of tetrahydrofuran, and 1.0 ml of water is added. Then, 302 mg (8.0 mM) of sodium borohydride is added with cooling with ice, and the mixture is allowed to react for 2 hours. After the reaction, 20 ml of 2N-hydrochloric acid is added. The tetrahydrofuran layer is separated, and the water layer is extracted with methylene chloride. The combined organic layers are dried and then evaporated to dryness under reduced pressure. The residue is subjected to silica gel column chromatography, and 1.27 g of 1,3-bis-5,8,11,14,17-eicosapentaenoylglyceride is obtained from the methylene chloride elution fraction. The physicochemical characteristics of this product are as follows.

IR_{max}^{KBr} (cm^{-1}): 3470, 3016, 1742

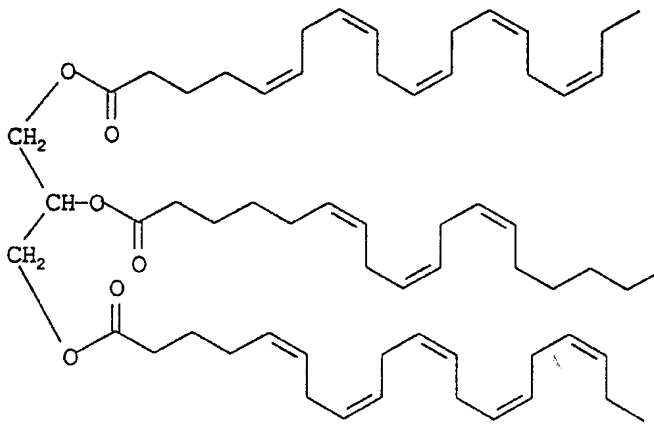
NMR ($CDCl_3$), δ(ppm): 0.97(6H,t,J=7.4Hz), 5.34(20H,m)

(3) 990 mg (1.5 mM) of the 1,3-bis-5,8,11,14,17-eicosapentaenoylglyceride obtained in (2), 417 mg (1.5 mM) of γ-linolenic acid and 18 mg (0.15 mM) of 4-dimethylaminopyridine are dissolved in 20 ml of methylene chloride, and a solution of 310 mg (1.5 mM) of N,N'-dicyclohexylcarbodiimide in 5.0 ml of methylene chloride is added dropwise under an atmosphere of nitrogen. The mixture is allowed to react at room temperature overnight. The reaction mixture is filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue is subjected to silica gel column chromatography, and 1,21 g of 1,3-bis-5,8,11,14,17-eicosapentaenoyl-2-6,9,12 octadecatrienoyl-glyceride is obtained from the methylene chloride elution fraction. The physicochemical data for this product are as follows.

IR_{max}^{KBr} (cm-1); 3016, 1746

NMR ($CDCl_3$), δ(ppm): 0.89(3H,t,J=7.0Hz), 0.97(6H,t,J=7.4Hz), 5.37(27H,m)
MASS (m/e): 920(M^+)

These physicochemical data support the structure of the following formula IX



IX

Other compounds of formula I (i.e. compounds of formulae II, III, IV, V, VI, VII, VIII and X) may also be prepared by processes including reduction of a 2-propane diglyceride, essentially as described in this Example.

Example 11 Activity as inhibitors of platelet aggregation.

1 Part by volume of 3.8% aqueous sodium citrate solution and 9 parts by volume of blood taken from the carotid arteries of a rabbit are mixed. The resultant mixture is centrifugally separated at room temperature to obtain platelet-enriched plasma (PRP: 200,000/µl).

99 µl of the above PRP is placed in a cuvette, and 1 µl of a 10% (w/v) solution of test compound in ether-ethanol (1:9) is added. The mixture is incubated at 37°C for 5 minutes, and 11 µl of an arachidonic acid solution is added to induce aggregation and permit measurement of platelet aggregation. Each test compound is assayed to determine the concentration required for 50% inhibition (IC_{50}) of the platelet aggregation induced by the arachidonic acid (300 µM). Table 1 shows the results. Aspirin and soybean oil are used as controls.

Table 1

Compound	Example	Concentration for 50% inhibition of platelet aggregation (mol)
II	1	7.6×10^{-5}
III	2	7.8×10^{-5}
IV	3	7.2×10^{-5}
V	4	6.0×10^{-5}
VI	5	6.6×10^{-5}
VII	6	6.5×10^{-5}
VIII	7	8.0×10^{-5}
IX	8	7.8×10^{-5}
X	9	7.6×10^{-5}
Aspirin (control)	-	2.5×10^{-5}
Soybean oil (control)	-	$>10^{-3}$

The above Table 1 clearly shows that the glyceride derivatives of the present invention had the activity for inhibiting platelet aggregation and that soybean oil had no activity.

Example 12 Anti-hypertriglyceridemia activity

Male Wistar rats (10 weeks of age) are orally administered with a CMS (0.5%) suspension of the compound of formula IX (1,3-dieicosapentaenyl-2-6,9,12-octadecatrienoyl glycerate). This oral administration is continued for 5 consecutive days. During the period of the oral administration, a synthetic high-cholesterol food shown in Table 2 is fed, whereby hypertriglyceridemia is caused. Blood samples are collected from the tail veins after 12 hours' fasting before the test and after 12 hours' fasting after the test. The measurement of serum triglyceride is carried out by an enzyme method. Table 3 shows the results.

Table 2

Synthetic cholesterol food*	(unit g)
Cholesterol	1.0
Bile acid	0.5
Milk casein	10.0
Salts	4.0
Hardened cottonseed oil	15.0
Choline chloride	0.2
p-Aminobenzoic acid	0.1
Inositol	0.1
Vitamin syrup	1.5
Sucrose	67.6
Total	100.0

* See Japan J. Pharmacol. Vol 23, pp. 289 to 298, 1973

Table 3

Group	Number of rats	Dose (g/kg)	Serum triglyceride concentration (mg/dl)		Average value ±standard error
			Before administration	After administration	Difference
Control	6	-	70.3	143.2	72.9±3.1
Compound [I]	6	1.3	69.3	116.1	46.7±6.2

As is clear in the above Table 3, it has been found that the compound of formula IX exhibits anti-hypertriglyceridemia activity with less than 1% significance in the t test.

Example 13 Preparation of Compositions

(1) Preparation of Soft capsules

Capsules each containing 200 mg of the compound of formula VI or the compound of formula IX are formed at room temperature using a rotary encapsulating machine. A gelatin capsule-forming recipe is used employing as the coating substrate 2.2 kg of gelatin, 0.66 kg of glycerin, 4.4 g of methylparaben, 1.1 g of propylparaben, 1.1 g of Yellow No. 5 and 1.8 kg of purified water.

(2) Preparation of Emulsion-1

400 Grams of the compound of formula II, 48 g of purified yolk lecithin, 2.0 g of oleic acid, 100 g of glycerin and 40 ml of 0.1N caustic soda are dispersed using a homomixer, and then distilled water for injection is added to a total volume of 4 liters. The above ingredients are emulsified in the distilled water for injection using an emulsifying machine. Emulsion-1 is used either as a platelet aggregation inhibitor or as a fat clysis preparation.

(3) Preparation of Emulsion-2

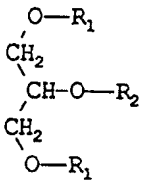
50 Grams of the compound of formula VII, 450 g of purified soybean oil, 60 g of purified yolk lecithin, 2.5 g of oleic acid, 125 g of glycerin and 50 ml of 0.1N caustic soda are dispersed with a homomixer, and then distilled water for injection is added to give a total volume of 5 liters. The above ingredients are emulsified in the distilled water for injection using an emulsifying machine. Emulsion-2 is used either as a platelet aggregation inhibitor or as a fat clysis preparation

Example 14 Acute toxicity

Male ICR mice (aged 5 weeks) are used for an acute toxicity test by oral administration. The compounds of the present invention, II, V, VII and IX have LD_{50} values of 5 g/kg or more, and thus have high safety.

CLAIMS

1. A compound of formula (I)



I

wherein R^1 and R^2 are acyl-groups derived from different unsaturated fatty acids both R^1 groups being identical, one acyl group of R^1 and R^2 is an acyl group derived from eicosapentaenoic acid or docosahexaenoic acid, and the other acyl group is an acyl group derived from linoleic acid, γ-linolenic acid, eicosapentaenoic acid or docosahexaneoic acid.

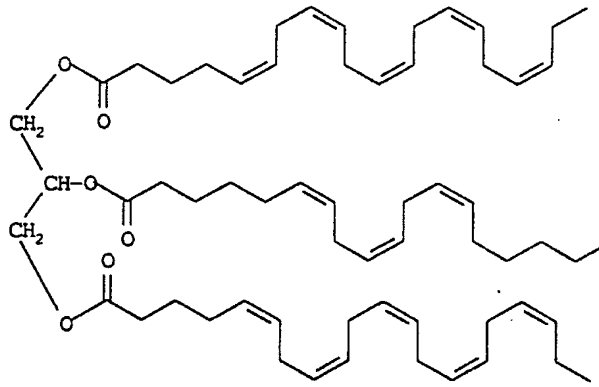
2. A compound of claim 1 in at least 90% pure form.

3. A compound of claim 1 containing less than 10% in total of other compounds of formula I.

4. The use of a compound of claim 1 as a pharmaceutical or a nutrient.

5. A compound according to claim 1 for use as a platelet aggregation inhibitor or as an anti-hypertriglyceridemic agent.

6. A compound of formula IX



IX

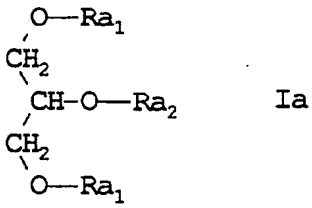
for use as an anti-hypertriglyceridemic agent.

7. A pharmaceutical or nutrient composition comprising a compound of claim 1.

8. A pharmaceutical composition according to claim 7 for use in the inhibition of platelet aggregation or as an anti-hypertriglyceridemic.

9. A parenteral nutrition composition comprising a compound of claim 1.

10. A process for the production of a compound of formula I as defined in claim 1 which comprises appropriately acylating a compound of formula Ia

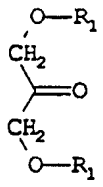


Ia

wherein Ra_1 is R_1 or hydrogen, both Ra^1 groups are identical and Ra_2 is R_2 or hydrogen, provided that one of Ra_1 and Ra_2

is other than hydrogen and R_1 and R_2 are as defined in claim 1.

11. A Process for the production of a compound of formula I as defined in claim 1, which comprises the step of reducing a 1,3-diacyl-2-propanone of formula Ib



Ib

where R_1 is as defined in claim 1, to obtain the corresponding 1,3-diacylglyceride.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 93/02917

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07C69/587 A61K31/23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 271 909 (GREEN CROSS CORPORATION) 22 June 1988 see column 2, line 52 - column 3, line 56 see column 6 - column 8; claims ---	1
A	PATENT ABSTRACTS OF JAPAN vol. 009, no. 281 (C-314)14 November 1985 & JP,A,60 132 916 (NISSHIN SEIYU KK) 16 July 1985 cited in the application see abstract ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 1994	26. 01. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kinzinger, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 93/02917

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	'CHEMICAL ABSTRACTS SERVICE.REGISTRY HANDBOOK.NUMBER SECTION.1988 SUPPLEMENT' 1988 , AMERICAN CHEMICAL SOCIETY , COLUMBUS,OHIO,US * RN = 115433-24-4 * see page 2694RQ * RN = 116198-42-6 * see page 3336RQ -----	1

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.formation on patent family members

International Application No
PCT/EP 93/02917

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0271909	22-06-88	JP-A- 63154618	27-06-88
		CA-A- 1292240	19-11-91